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Stable carbon and nitrogen isotope variations in tooth dentine serial sections from Wharram Percy

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Abstract

Here we report $\delta^{13}C$ and $\delta^{15}N$ measurements of serial sections of human deciduous and permanent tooth dentine from archaeological samples taken from the medieval village site of Wharram Percy, Yorkshire, UK. We found a pattern of enrichment, for both $\delta^{13}C$ and $\delta^{15}N$, where the tooth crown was greater than the cervical part of the root, which in turn was greater than the apical portion of the root and the associated rib collagen values. This pattern reflects a decrease in the consumption of isotopically enriched breast milk and the introduction of less enriched weaning foods in the diet. The (mean ± SD) difference between the deciduous second molar crowns and corresponding rib samples from the same individuals after 2 years of age was 1.2 ± 0.4‰ for $\delta^{13}C$ and 3.2 ± 0.8‰ for $\delta^{15}N$. The $\delta^{15}N$ values are as predicted, but as there were no C_4 plants at Wharram Percy, this 1.2‰ enrichment in $\delta^{13}C$ represents clear evidence of a carbon trophic level effect in collagen from breastfeeding infants. Carbon and nitrogen results also show that the infant diet among those who died in infancy did not differ from those who survived into childhood. This study demonstrates the promise of using dentine serial sections to study the temporal relationships of breastfeeding, weaning, and dietary patterns of single individuals.

Keywords: Weaning; Palaeodiet; Dentine; Stable isotopes; Carbon; Nitrogen; Wharram Percy

1. Introduction

The duration of breastfeeding and the weaning process (the introduction of solid foods to an infant's diet) have increasingly become a focus of investigation in archaeological populations since the stable isotope ratio of nitrogen ($\delta^{15}N$) in body tissues can be used as a natural tracer to identify the consumption of breast milk [13,18,22,23,28,31,34,35,40,41]. Breastfeeding children have tissue $\delta^{15}N$ values 2–3‰ higher than their mothers [14] as a result of the "trophic level effect" [32] where consumer tissue $\delta^{15}N$ values are elevated by approximately 2–4‰ over dietary protein. During the weaning process, the consumption of supplementary foods results in a decline in infant $\delta^{15}N$ values. When a child is fully weaned (cessation of breastfeeding), its protein $\delta^{15}N$ values are nearly identical to those of its mother, assuming similar diets [14].

For isotopic studies, the age of weaning in archaeological collections has traditionally been estimated by measuring the $\delta^{15}N$ of bone collagen (ribs) from different age classes of infants and children and noting the age at which the $\delta^{15}N$ values return to those of the adult population. While this method yields a general timeframe for the duration of breastfeeding, a precise calculation of the introduction of weaning foods is not possible, as uncertainty exists about the amount of time needed for the infant skeleton to fully incorporate the isotopically depleted post-weaning collagen [36,39]. In addition, $\delta^{15}N$ values obtained from infants who died during breastfeeding and weaning need to be interpreted with caution as these individuals might have been fed different diets or died of malnutrition, and this has the potential to alter the expected $\delta^{15}N$ values [20].

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Table 1a
Estimated age-spans of formation in tooth dentine serial sections

Teeth (3 sections)	Approximate timing of dentine development		
	Crown	Cervical root half	Apical root half
Deciduous M2	6 months in utero–11 months post-natal	11 months–2 years	2–3 years
Permanent canine	5 months–6 years	6–10 years	10–14 years
Permanent M3	9.5–13.5 years	13.5–16.7 years	16.7–20 years

Table 1b

Teeth (4 sections)	Crown	Cervical root third	Middle root third	Apical root third
Permanent canine	5 months–6 years	6–8.7 years	8.7–11.4 years	11.4–14 years
Permanent M3	9.5–13.5 years	13.5–15.7 years	15.7–17.8 years	17.8–20 years

Note: Dentine development schedule derived from Gustafson and Koch [17], except for third molars, which use data collated from Anderson et al. [2], Levesque et al. [25] and Garn et al. [16].

The serial sectioning of human skeletal tissues, enamel and dentine, that are metabolically inert to remodeling [3,15] could circumvent the problems mentioned above and allow the study of weaning and dietary habits at a more refined level. While a number of studies have measured bulk isotopic ratios from dentine collagen [4,6–8,31,38,41] and noted an offset with bone collagen, little research has been conducted on dentine serial sections. Pioneering research on dentine serial sections from Stellar sea lions showed that isotopic signals were preserved along annual growth lines and recorded the dietary and environmental conditions of the animal [21]. Drucker et al. [12] used serial sections from the tooth roots of modern caribou to examine short-term dietary changes and lichen consumption, and a study of dentine serial sections in modern cattle teeth by [5] assessed the duration of weaning and examined a change from a C_3 to C_4/C_3 diet. In this paper, we present $\delta^{13}C$ and $\delta^{15}N$ measurements of human dentine collagen serial sections from deciduous second molars, permanent canines and third molars. These skeletal samples are from the medieval site of Wharram Percy, Yorkshire, UK. The dentine collagen results are then compared to rib collagen $\delta^{13}C$ and $\delta^{15}N$ values from the same individual to gain a fuller understanding of individual isotopic life histories.

2. Materials

Located in the Yorkshire Wolds about 25 miles east of the modern city of York, the deserted medieval village of Wharram Percy is one of the most thoroughly excavated and best preserved English sites of the time period. The Wharram Percy human skeletal collection consists of 687 individuals and was chosen for weaning studies since nearly half of the assemblage represents individuals under the age of 18. In addition, the individuals recovered from the church and churchyard represent a geographically defined and socially meaningful population since only those inhabitants of this rural parish were buried there [26]. The ribs and teeth used in this research date primarily from the 10th–16th century.

3. Methods

Deciduous second molars (n=21), permanent third molars (n=8), permanent canines (n=8), and corresponding rib samples were analyzed for this study. Age at death for the juveniles sampled was determined using dental development [33]; for the adults age was estimated using dental wear [27,29]. All teeth studied were free of caries and were first shot blasted to remove surface debris (soil) and then vertically sectioned in half with a low speed diamond saw. One of the tooth halves was then cut horizontally while the other was retained for future analysis. Except in cases of incomplete tooth formation, deciduous second molars were sectioned into three parts corresponding to the crown (defined as the dentine enclosed by the enamel), and the cervical and apical halves of the root. For the permanent third molars, six of the eight teeth were also cut into crown, and cervical and apical halves of the root. The two remaining third molars (EE36 and EE3) were slightly larger and were sectioned into four portions corresponding to the crown, and cervical, middle and apical thirds of the root. As a result of their greater length, the permanent canines were cut into four portions corresponding to the crown, and cervical, middle, and apical thirds of the root, except for G597 which was cut into three portions comprising the crown and the cervical and apical root halves. The estimated ages at formation of the dentine serial sections are shown in Tables 1a and 1b. We are aware that tooth dentine does not form horizontally, but the analytical equipment we used

Table 2
$\delta^{13}C$ and $\delta^{15}N$ results for dentine serial sections from deciduous second molars and corresponding rib samples. Measurement errors= ± 0.2‰ for $\delta^{13}C$ and ± 0.3‰ for $\delta^{15}N$

Sample	Serial section	Age (years)	$\delta^{13}C$	$\delta^{15}N$	C:N
G522	crown	1.3	−19.0	12.1	3.17
G522	rib	1.3	−19.8	11.6	3.20
NA37	crown	1.3	−19.6	10.0	3.20
NA37	rib	1.3	−20.1	9.8	3.23
G327	crown	1.5	−18.6	12.2	3.10
G327	cervical root	1.5	−18.6	12.9	3.11
G327	rib	1.5	−19.1	12.3	3.17
NA28	crown	1.5	−18.5	13.3	3.20
NA28	rib	1.5	−18.9	13.1	3.30
G430	crown	1.8	−19.6	11.1	3.20
G430	cervical root	1.8	−19.8	9.4	3.05
G430	rib	1.8	−20.4	9.8	3.18
WCO72	crown	2	−19.0	9.8	3.14
WCO72	cervical root	2	−19.0	9.3	3.22
WCO72	rib	2	−19.6	8.5	3.25
G339	crown	2.5	−19.6	12.3	3.14
G339	cervical root	2.5	−19.9	10.0	3.04
G339	apical root	2.5	−19.7	9.3	3.13
G339	rib	2.5	−20.4	8.1	3.21
G363	crown	2.5	−18.7	11.6	3.15
G363	cervical root	2.5	−19.0	10.6	3.17
G363	rib	2.5	−20.0	8.8	3.24
NA79	crown	3	−19.0	10.0	2.99
NA79	cervical root	3	−19.1	10.8	3.18
NA79	apical root	3	−19.6	9.6	3.23
NA79	rib	3	−20.2	8.5	3.23
G576	crown	3.3	−19.4	11.6	3.05
G576	cervical root	3.3	−19.5	11.1	3.18
G576	rib	3.3	−20.5	9.3	3.22
WCO97	crown	5	−18.3	11.5	3.16
WCO97	cervical root	5	−18.5	11.1	3.16
WCO97	apical root	5	−18.6	8.4	3.17
WCO97	rib	5	−19.4	7.5	3.17
G614	crown	5.5	−19.1	11.3	3.12
G614	cervical root	5.5	−19.4	10.0	3.18
G614	rib	5.5	−20.5	8.5	3.14
NA30	crown	6	−18.6	12.2	3.16
NA30	cervical root	6	−19.2	10.6	3.19
NA30	apical root	6	−19.4	9.7	3.16
NA30	rib	6	−20.7	8.0	3.35
G424	crown	6.5	−18.8	12.1	3.14
G424	cervical root	6.5	−19.1	11.6	3.19
G424	apical root	6.5	−19.7	9.9	3.21
G424	rib	6.5	−19.9	8.4	3.20
NA23	crown	7	−19.1	11.6	3.18
NA23	cervical root	7	−19.4	10.9	3.19
NA23	apical root	7	−19.5	8.6	3.19
NA23	rib	7	−19.8	8.4	3.21
EE65	crown	7.5	−19.3	11.0	3.17
EE65	cervical root	7.5	−19.8	9.9	3.21
EE65	apical root	7.5	−20.0	9.1	3.22
EE65	rib	7.5	−20.4	7.3	3.08
EE66	crown	8.5	−18.7	12.8	3.19
EE66	cervical root	8.5	−19.4	11.7	3.18
EE66	apical root	8.5	−19.9	9.9	3.25
EE66	rib	8.5	−20.1	10.1	3.23
WCO140	crown	9	−18.6	12.4	3.16
WCO140	cervical root	9	−18.8	12.2	3.21
WCO140	apical root	9	−19.5	8.5	3.06
WCO140	rib	9	−19.9	8.8	3.22
G500	crown	9.5	−18.9	12.0	3.17
G500	cervical root	9.5	−19.6	10.4	3.17
G500	apical root	9.5	−19.7	9.6	3.19
G500	rib	9.5	−20.1	8.7	3.25
EE72	crown	10	−19.4	12.4	3.21
EE72	cervical root	10	−19.6	10.8	3.20
EE72	apical root	10	−20.2	9.5	3.31
EE72	rib	10	−20.1	9.0	3.32
G658	crown	11	−18.1	10.3	3.14
G658	cervical root	11	−18.6	9.9	3.18
G658	apical root	11	−20.0	9.0	3.54
G658	rib	11	−19.7	7.9	3.18

required relatively large samples of collagen (3–5 mg), and therefore we had to compromise between accurately sampling dentine along growth increment lines with obtaining enough sample for analysis.

Rib and tooth samples were prepared for isotopic analysis at the Research Laboratory for Archaeology and the History of Art, University of Oxford. Collagen was extracted from the rib samples and the teeth serial sections following a modified Longin procedure, as outlined in detail elsewhere [30]. As the crown serial sections were encased in enamel, the demineralization process was much slower and took approximately 4–7 days to complete. The resultant solids from the ribs and the teeth were gelatinized in pH 3 water at 70 °C for 48 h and the final solutions filtered and lyophilized. This process resulted in the extraction of a number of bone and tooth proteins, but the majority of the extracted material was bone and dentine collagen. The collagen was placed in tin capsules and combusted to CO_2 and N_2 in an automated carbon and nitrogen analyzer (Carlo Erba) coupled to a continuous-flow isotope ratio monitoring mass spectrometer (PDZ Europa Geo 20/20). Replicate measurement errors are less than ± 0.2‰ for $\delta^{13}C$ and ± 0.3‰ for $\delta^{15}N$. All results are reported in units of per mil (‰) with $\delta^{13}C$ relative to VPDB (Vienna Pee Dee Belemnite), and with $\delta^{15}N$ relative to AIR (Ambient Inhalable Reservoir).

4. Results and discussion

4.1. Deciduous dentition

The stable isotope values for the deciduous second molar serial sections and corresponding ribs from the same individual are presented in Table 2. Deciduous second molars were chosen to study breastfeeding and weaning since the dentine formation in this tooth spans the period from about 6 months in-utero until root completion at approximately 3 years of age (Table 1a). Complete serial sectioning (crown and cervical/apical halves of the root) of teeth under 3 years of age was not

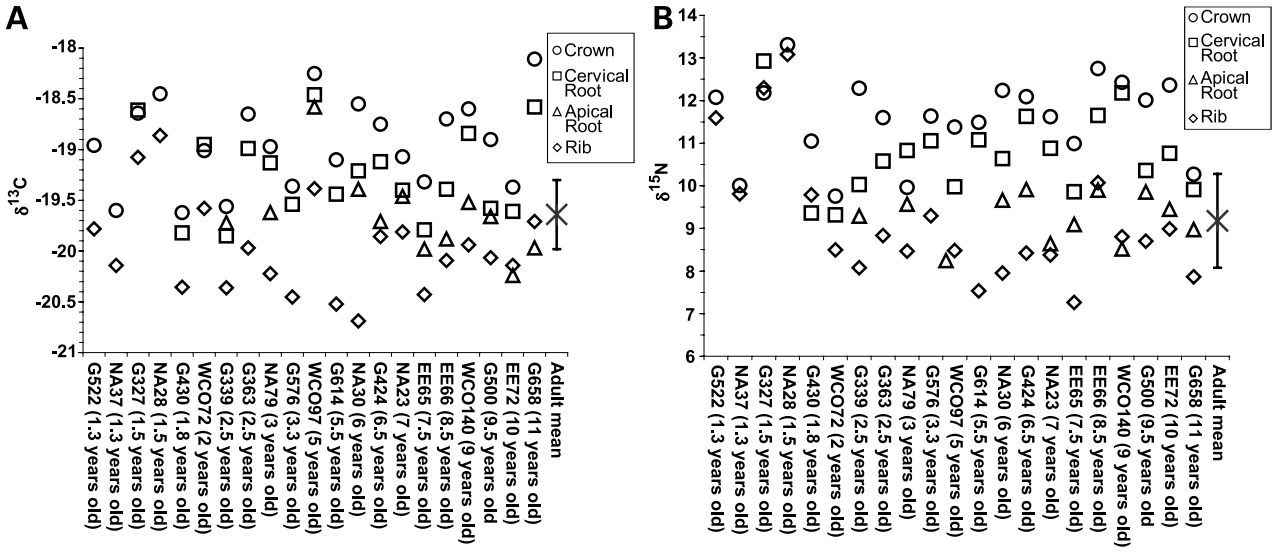


Fig. 1. $\delta^{13}C$ (a) and $\delta^{15}N$ (b) values for dentine serial sections from deciduous second molars and ribs plotted for individual samples. Also shown is the adult mean ± SD.

possible due to incomplete tooth formation. Thus, for these teeth the crown was separated from as much of the root as had formed. All the teeth and ribs sampled had collagen yields between 4% and 15% and had C/N ratios within the expected range for collagen of 2.9 to 3.6 [10], indicating good preservation of the skeletal material.

The $\delta^{13}C$ values of the deciduous second molars are plotted in Fig. 1a. For nearly all individuals, the following ^{13}C enrichment pattern is observed: crown>cervical half of root>apical half of root>rib. Two infants (G327, WCO72) have crown and cervical root portion $\delta^{13}C$ values that are essentially equal, and the apical half of the root from G339 is slightly enriched compared to the cervical portion but this difference is within the analytical error (±0.2‰) for carbon. In addition, the two oldest children (EE72, G658), aged 10 and 11 years, have rib $\delta^{13}C$ values that are more enriched than the apical halves of the deciduous second molar roots by 0.1‰ and 0.3‰, respectively. Again, these $\delta^{13}C$ values are within the analytical error, and thus it is impossible to interpret the results as reflecting slight variations in dietary carbon in later life for these individuals.

The $\delta^{15}N$ values for the deciduous second molars are plotted in Fig. 1b. The ^{15}N enrichment pattern was the same as for carbon (crown>cervical half of root>apical half of root>rib). That the crown dentine generally shows greater ^{15}N enrichment than the cervical half of the root indicates that a relatively greater proportion of the crown dentine formed during the time the infant was breastfeeding, despite the fact that it incorporates a small amount of dentine which formed before birth. Given the developmental timing of these parts of the tooth (Table 1a), this pattern is as expected if, as inferred from previous work [28,31], breastfeeding was normally discontinued after about 18 months post-partum as a significant part of the cervical tooth root sample would have formed after this. There were some variations in this general pattern. Individual G430 (1.5–2 years) has a rib $\delta^{15}N$ value that is elevated by 0.4‰ compared to the cervical portion of the deciduous second molar root, and two children (EE66, WCO140) have rib values that are slightly enriched in ^{15}N. In particular, one infant (G327) aged about 1.5 years has a $\delta^{15}N$ value for the cervical portion of the deciduous second molar root that is 0.8‰ elevated over that of the crown. The higher value for the cervical part of the (incomplete) tooth root is as expected if this individual was breast-fed until time of death, as under these circumstances all of the (incompletely formed) root sample would have formed during the period this individual was breastfeeding, whereas, as discussed above, a small proportion of the crown dentine forms prior to birth and so would be relatively depleted in ^{15}N.

The 3 year old (NA79) shows the same ^{15}N enrichment as G327, cervical half of root>crown. However, in this case the low crown $\delta^{15}N$ value (10‰) points to the fact that this child was not exclusively breastfed or had difficulty breastfeeding soon after birth. This low consumption of breast milk in the early diet appears to have been increased in later life since the cervical half of the root is enriched in ^{15}N by 0.9‰ compared to the crown. The crown $\delta^{15}N$ values for NA37 and WCO72 are 10‰ and 9.8‰, respectively (Fig. 1b). These results are well below the mean of the other crown sections (11.6 ± 1.0‰; mean ± SD) and could suggest that these individuals were not or only minimally breastfed. As breastfeeding has been proven to confer important

nutritional and health benefits to infants such as a decreased risk of infection, diarrhea, and mortality [9,11,24], this possible lack of breastfeeding for NA37 and WCO72 might have contributed to their early deaths. The 11 year old (G658) also displays a low crown δ^{15}N value (10.3‰) (Fig. 1b). While the difference in δ^{15}N between the crown and the rib (2.4‰) indicates that this individual was breastfed, the low crown values suggests that some supplementary foods were added to the diet at an earlier age thereby diluting the full ^{15}N enrichment of the breast milk. It is also possible that the low crown δ^{15}N value reflects the fact that the diet and thereby the breast milk from the mother of this infant was lower in δ^{15}N compared to the rest of the population.

Most studies of the duration of breastfeeding in past populations use δ^{15}N values from bone collagen. Such studies, involve determining δ^{15}N values from infants and children who died over a range of different ages. Weaning practices are therefore reconstructed from children who failed to survive. Breastfeeding practices as reconstructed from those who died in infancy may not wholly typify regimes for those who did survive infancy but rather they may identify unsuccessful strategies that increased risk of infant death. To a limited extent, the current data permit investigation of whether those who died in infancy at Wharram Percy generally experienced different breast-feeding practices to those who survived into later childhood.

Dentine formation in the crown of the deciduous second molar begins at about 6 months in-utero, and the crown is complete by about 11 months after birth (Table 1a). Previous research [31] has determined that breast-feeding continued for 1–2 years after birth in this population. Thus, most of the second molar crown dentine formed at a time during which most infants were being breastfed at Wharram Percy. Our hypothesis is that if infant feeding/weaning practices differed between those who died aged 2 or under and those who survived beyond 2 years (i.e. beyond what we believe to be the normal duration of breastfeeding), then the δ^{13}C and δ^{15}N values of the crown dentine might also differ between these two age groups.

Carbon and nitrogen stable isotope results from deciduous second molars for 6 individuals aged 2 or under and 15 individuals aged over 2 years are listed in Table 2. Through the use of *t*-tests, no significant difference in either δ^{13}C or δ^{15}N was determined for the two age groups (Table 3). Although the numbers are small, the current data offer no support for the notion that, at Wharram Percy, infant diet among those who died in infancy generally differed from that of those who survived.

The mean δ^{13}C and δ^{15}N results for the deciduous second molar serial sections and ribs are presented in Fig. 2. This data is only plotted for individuals aged

Table 3
δ^{13}C and δ^{15}N values (‰) in those aged 2 years or less (N=6) and those aged over 2 years at death (N=15)

	Age group	Mean	SD
δ^{13}C	<=2 years	19.1	0.5
	>2 years	18.9	0.4
δ^{15}N	<=2 years	11.4	1.4
	>2 years	11.7	0.8

t-tests <=2 years vs. >2 years: δ^{13}C, t=0.7; δ^{15}N, t=0.5. In each case $P \sim 0.2$ at 19 degrees of freedom, i.e. non-significant.

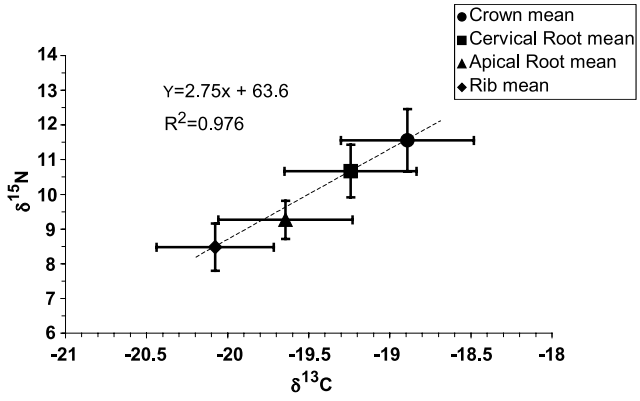


Fig. 2. δ^{13}C and δ^{15}N results (mean ± SD) for all deciduous second molar dentine serial sections: crown, cervical root half, apical root half and rib samples from individuals greater than 2 years old.

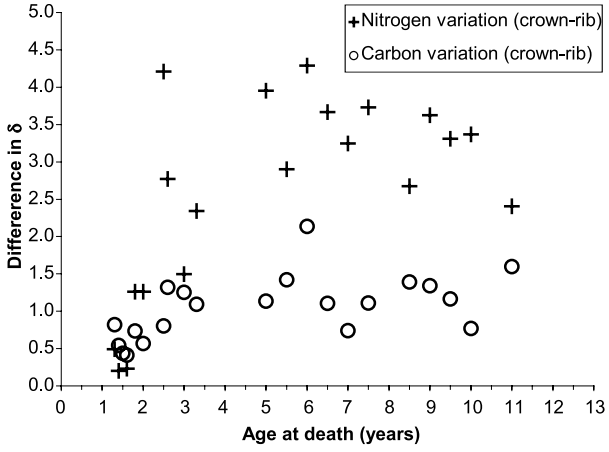


Fig. 3. δ^{13}C and δ^{15}N differences between the deciduous second molar crowns and ribs plotted against age of death.

greater than two years so that the magnitude of the dietary transition from breast milk to weaning foods can be assessed. A depletion pattern (mean ± SD) with a very strong linear correlation (R^2=0.976) is observed (crown>cervical half of root>apical half of root>rib) in both carbon (-18.9 ± 0.4‰ > -19.3 ± 0.4‰ > -19.7 ± 0.4‰ > -20.1 ± 0.4‰) and nitrogen (11.7 ± 0.8‰ > 10.8 ± 0.7‰ > 9.3 ± 0.5‰ > 8.5 ± 0.7‰). This dual

Table 4
$\delta^{13}C$ and $\delta^{15}N$ results for dentine serial sections from permanent canines (C), permanent third molars (M3) and corresponding rib samples. Measurement errors= ± 0.2‰ for $\delta^{13}C$ and ± 0.3‰ for $\delta^{15}N$

Sample	Serial section	Age (years)	Sex	$\delta^{13}C$	$\delta^{15}N$	C:N
EE36 (C)	crown	18–21	Female	−19.0	9.9	3.18
EE36 (C)	cervical root	18–21	Female	−19.3	8.9	3.19
EE36 (C)	middle root	18–21	Female	−18.3	8.4	3.17
EE36 (C)	apical root	18–21	Female	−18.8	8.5	3.24
EE36 (M3)	crown	18–21	Female	−18.7	9.1	3.17
EE36 (M3)	cervical root	18–21	Female	−19.0	7.9	3.13
EE36 (M3)	middle root	18–21	Female	−19.0	8.0	3.16
EE36 (M3)	apical root	18–21	Female	−18.6	8.4	3.19
EE36	rib	18–21	Female	−19.2	9.0	3.29
CN28 (C)	crown	22–24	Female	−19.4	8.0	3.03
CN28 (C)	cervical root	22–24	Female	−19.6	8.1	3.15
CN28 (C)	middle root	22–24	Female	−19.6	7.3	3.14
CN28 (C)	apical root	22–24	Female	−19.2	7.8	3.19
CN28 (M3)	crown	22–24	Female	−19.4	8.1	3.16
CN28 (M3)	cervical root	22–24	Female	−19.2	8.7	3.21
CN28 (M3)	apical root	22–24	Female	−19.0	9.2	3.20
CN28	rib	22–24	Female	−19.4	9.5	3.26
G597 (C)	crown	22–25	Female	−19.4	9.3	3.18
G597 (C)	cervical root	22–25	Female	−19.4	7.6	3.14
G597 (C)	apical root	22–25	Female	−19.6	7.7	3.19
G597 (M3)	crown	22–25	Female	−19.5	8.2	3.16
G597 (M3)	cervical root	22–25	Female	−19.1	8.2	3.18
G597 (M3)	apical root	22–25	Female	−19.0	8.9	3.22
G597	rib	22–25	Female	−19.9	7.8	3.28
CN2 (C)	crown	22–25	Female	−19.6	11.7	3.06
CN2 (C)	cervical root	22–25	Female	−19.3	11.2	3.16
CN2 (C)	middle root	22–25	Female	−19.5	10.6	3.19
CN2 (C)	apical root	22–25	Female	−19.3	10.6	3.16
CN2 (M3)	crown	22–25	Female	−19.5	11.2	3.16
CN2 (M3)	cervical root	22–25	Female	−19.3	10.0	3.13
CN2 (M3)	apical root	22–25	Female	−19.3	10.5	3.26
CN2	rib	22–25	Female	−19.4	10.5	3.13
EE3 (C)	crown	25–35	Female	−20.0	11.0	3.19
EE3 (C)	cervical root	25–35	Female	−19.7	8.6	3.15
EE3 (C)	middle root	25–35	Female	−19.5	8.0	3.17
EE3 (C)	apical root	25–35	Female	−19.5	8.0	3.17
EE3 (M3)	crown	25–35	Female	−19.1	9.4	3.13
EE3 (M3)	cervical root	25–35	Female	−20.7	9.0	3.12
EE3 (M3)	middle root	25–35	Female	−19.7	10.8	3.23
EE3 (M3)	apical root	25–35	Female	−20.0	8.6	3.39
EE3	rib	25–35	Female	−19.8	10.3	3.29
NA59 (C)	crown	35–45	Male	−19.5	9.8	3.16
NA59 (C)	cervical root	35–45	Male	−19.8	9.2	3.17
NA59 (C)	middle root	35–45	Male	−19.6	9.3	3.23
NA59 (C)	apical root	35–45	Male	−20.0	8.6	3.18
NA59 (M3)	crown	35–45	Male	−19.9	9.2	3.15
NA59 (M3)	cervical root	35–45	Male	−19.6	9.5	3.20
NA59 (M3)	apical root	35–45	Male	−19.8	9.9	3.20
NA59	rib	35–45	Male	−20.1	8.9	3.12
EE67 (C)	crown	30–50	Male	−19.6	8.3	3.07
EE67 (C)	cervical root	30–50	Male	−19.1	9.3	3.19
EE67 (C)	middle root	30–50	Male	−19.3	9.4	3.18
EE67 (C)	apical root	30–50	Male	−19.2	9.5	3.20
EE67 (M3)	crown	30–50	Male	−19.4	9.8	3.17
EE67 (M3)	cervical root	30–50	Male	−19.2	9.5	3.14
EE67 (M3)	apical root	30–50	Male	−18.5	10.4	3.17
EE67	rib	30–50	Male	−19.7	9.1	3.27
G746 (C)	crown	30–50	Female	−19.4	9.6	3.15
G746 (C)	cervical root	30–50	Female	−19.2	9.3	3.16
G746 (C)	middle root	30–50	Female	−19.3	8.8	3.12
G746 (C)	apical root	30–50	Female	−19.6	8.7	3.18

Table 4 (*continued*)

Sample	Serial section	Age (years)	Sex	$\delta^{13}C$	$\delta^{15}N$	C:N
G746 (M3)	crown	30–50	Female	−19.8	9.2	3.17
G746 (M3)	cervical root	30–50	Female	−19.7	8.7	3.18
G746 (M3)	apical root	30–50	Female	−19.2	9.0	3.17
G746	rib	30–50	Female	−19.4	9.6	3.23

sequence of enrichment indicates that infants display both a carbon and nitrogen trophic level shift during breastfeeding. The mean ^{15}N enrichment of the crown dentine over the ribs is 3.2 ± 0.8‰ (Fig. 3). This observed 3.2‰ enrichment is in good agreement with the results of previous studies of infant bone collagen [13,18,22,23,34]. In addition, the mean ^{13}C enrichment of the crown dentine over the ribs is 1.2 ± 0.4‰ (Fig. 3). Enrichment in ^{13}C (0.5–1.4‰) has been previously noted in infant collagen by other researchers [13,22,23,31,41] but never with the consistency and clarity of the present results (Fig. 3). Since many of these past studies were conducted in regions where there is an input of both C_3 and C_4 plants, this increase in $\delta^{13}C$ has sometimes been interpreted as infants being weaned onto C_4 dietary components such as maize gruel or milk from animals fed millet. In addition, the only longitudinal study of modern breastfeeding infants did not find a change in $\delta^{13}C$ during suckling [14]. Thus, there has been confusion about the existence of this carbon trophic level effect and its exact magnitude for breastfeeding infants.

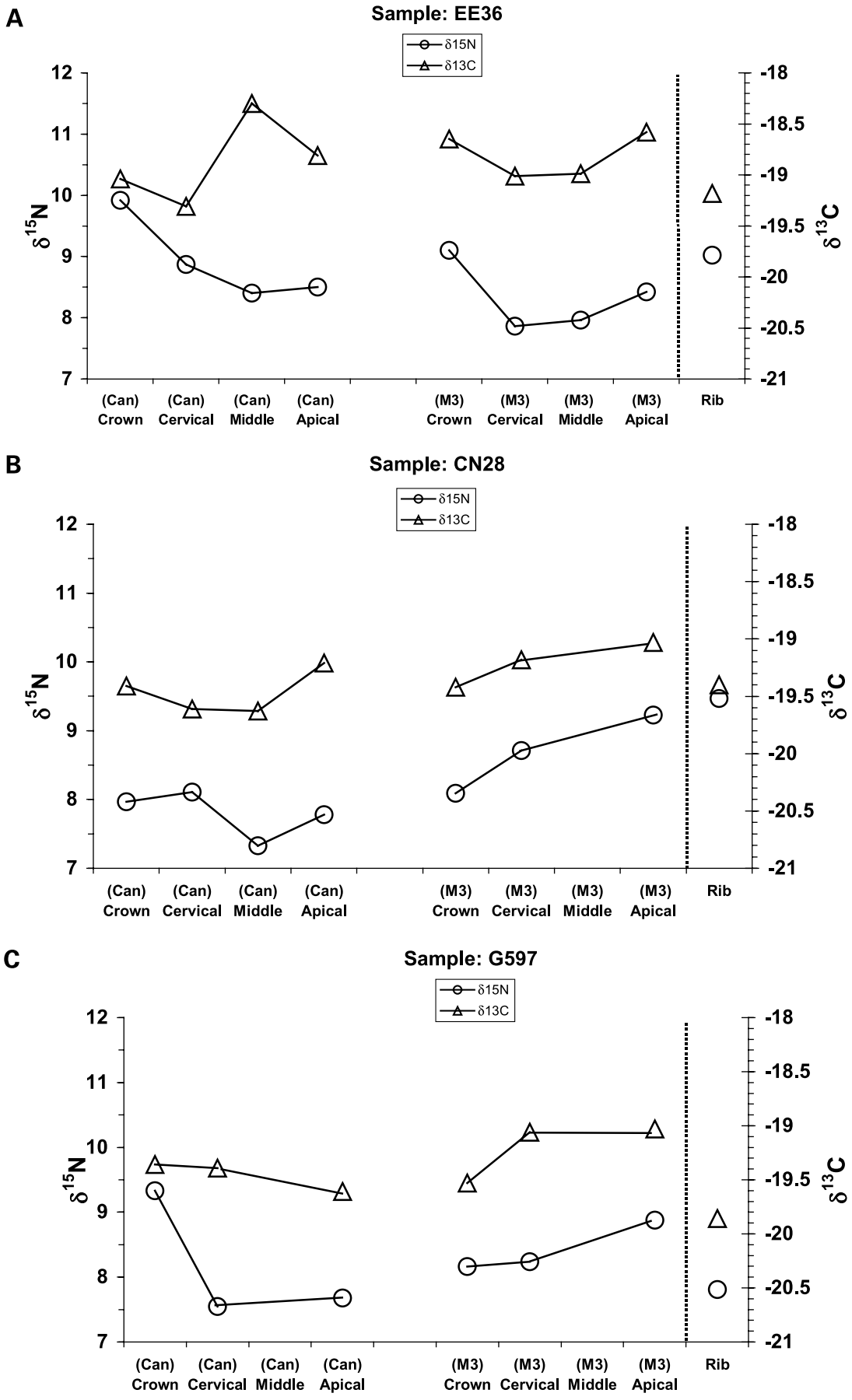
The data from Wharram Percy clearly show a change in both $\delta^{13}C$ and $\delta^{15}N$ from the crown to the apical root of the second molar teeth. Since Wharram Percy had no known C_4 plants that could be used as supplements to breast milk during weaning, the data presented here (Fig. 3) strongly suggests that the 1.2‰ enrichment in ^{13}C is entirely the result of a carbon trophic level effect [32] of breastfeeding infants. Thus, it is likely that the observed ^{13}C enrichment in infant skeletons from previous studies [13,22,23,41] are the result of a breast milk trophic level effect and not due to the input of C_4 weaning foods. Ultimately, this debate over the existence of a ^{13}C enrichment in the protein of breastfeeding infants will have to be resolved by modern human longitudinal studies, which are currently underway. Preliminary results do indeed suggest a 1‰ ^{13}C enrichment in the hair and nails of breastfeeding infants compared to maternal $\delta^{13}C$ values (Fuller et al., in prep.).

4.2. Permanent dentition

The results for the permanent canines, permanent third molars and ribs are presented in Table 4. The timing of the development of these teeth means that the majority of the dentine in the canine, and all of the dentine in the third molar, was laid down after the cessation of breastfeeding. As there is significant overlap in the formation of the permanent canines and permanent third molars, it is expected that the isotopic signatures will track each other during these periods, and this is generally observed in the results (Fig. 4 a–h). These specific teeth were chosen as a pilot study to examine the possibility of tracking changing individual dietary habits during childhood and adolescence. All the teeth and ribs examined had collagen yields between 4% and 15% and had C/N ratios within the expected range for collagen of 2.9 to 3.6 [10].

The $\delta^{13}C$ results for the permanent canines, permanent third molars, and ribs from the same individuals are plotted in Fig. 4a–h. Unlike the deciduous second molars, no discrete patterning was observed between the serial sections of the canines and the third molars for $\delta^{13}C$. The majority of the collagen $\delta^{13}C$ values (−19‰ to −20‰) indicate a diet based largely on C_3 plant and terrestrial animal protein with little input from marine protein sources [36]. As there are only small variations in the $\delta^{13}C$ values, the sources of dietary carbon were relatively constant during the lifetime of these individuals. However, the female (EE36) shows a ^{13}C enrichment in her middle (−18.3‰) and apical (−18.8‰) canine roots and in her third molar crown (−18.7‰) which could suggest some marine food consumption during late childhood or early adolescence (Fig. 4a). In addition, the male (EE67) also displays increases in both $\delta^{13}C$ (−18.5‰) and $\delta^{15}N$ (10.4‰) for the apical half of the third molar root (Fig. 4g). Since $\delta^{13}C$ and $\delta^{15}N$ values are elevated in marine ecosystems [36], this dual enrichment might be the result of a small input of marine protein to the diet of this male during his late teenage years. As the consumption of marine food was minor in the Wharram Percy population, it is possible that these individuals travelled and lived near the coast during this time. The crown and the cervical third of the third molar root from EE3 show the largest variation in $\delta^{13}C$ (1.6‰), suggesting that the diet of this individual was more variable during early adolescence (Fig. 4e).

Fig. 4a–h also illustrate the $\delta^{15}N$ values for permanent canines, permanent third molars, and ribs from the same individuals. In contrast to the $\delta^{13}C$ results, the $\delta^{15}N$ values show much more variation in both the canines and the third molars. This reflects the different dietary protein sources where the enrichment in ^{15}N of collagen is primarily the result of an increase in animal


A
Sample: EE36
δ15N
δ13C
δ15N
δ13C
12
11
10
9
8
7
-18
-18.5
-19
-19.5
-20
-20.5
-21
(Can) Crown
(Can) Cervical
(Can) Middle
(Can) Apical
(M3) Crown
(M3) Cervical
(M3) Middle
(M3) Apical
Rib
B
Sample: CN28
δ15N
δ13C
δ15N
δ13C
12
11
10
9
8
7
-18
-18.5
-19
-19.5
-20
-20.5
-21
(Can) Crown
(Can) Cervical
(Can) Middle
(Can) Apical
(M3) Crown
(M3) Cervical
(M3) Middle
(M3) Apical
Rib
C
Sample: G597
δ15N
δ13C
δ15N
δ13C
12
11
10
9
8
7
-18
-18.5
-19
-19.5
-20
-20.5
-21
(Can) Crown
(Can) Cervical
(Can) Middle
(Can) Apical
(M3) Crown
(M3) Cervical
(M3) Middle
(M3) Apical
Rib

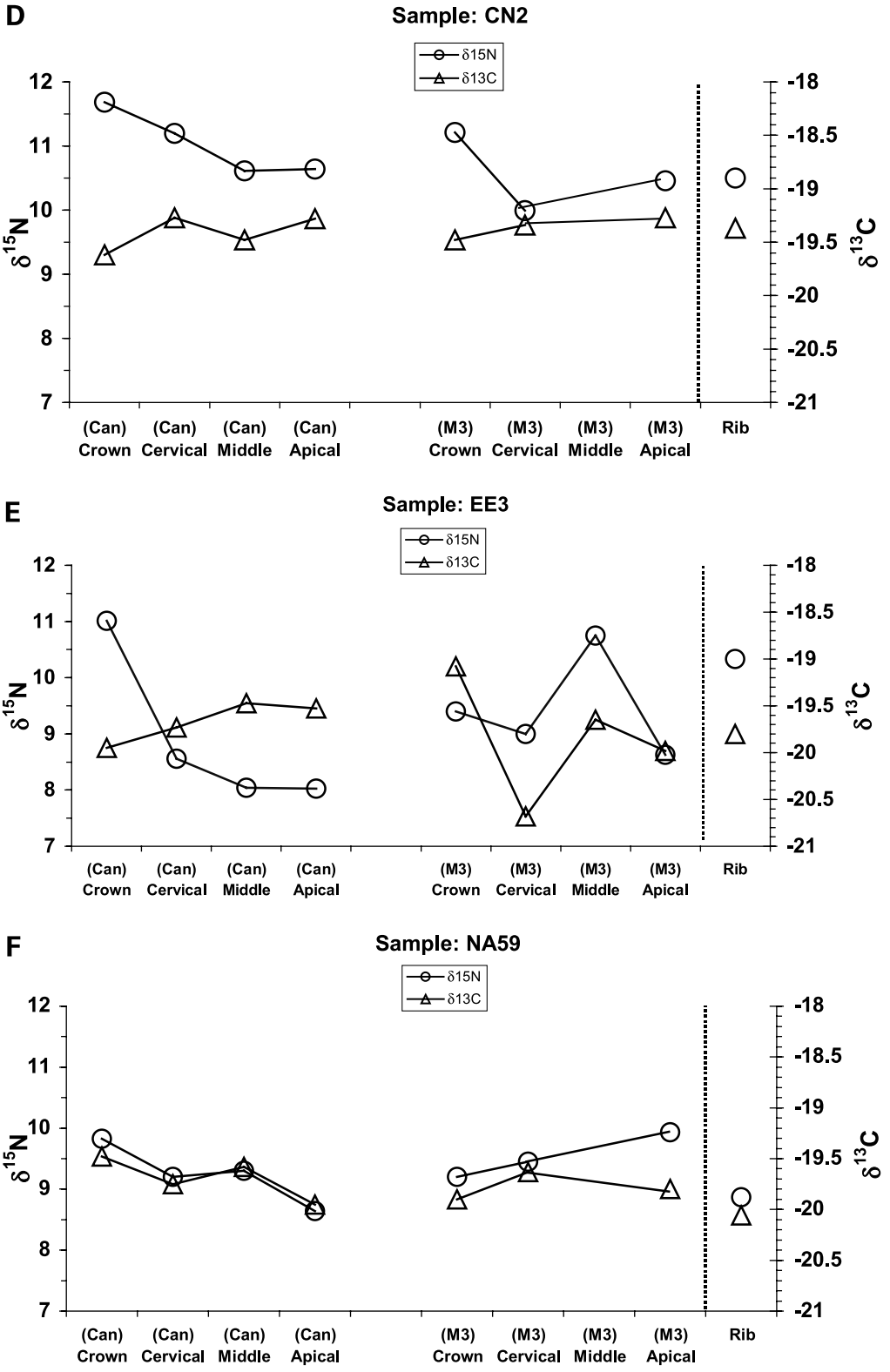

D
Sample: CN2
δ15N
δ13C
δ15N
δ13C
12
11
10
9
8
7
-18
-18.5
-19
-19.5
-20
-20.5
-21
(Can) Crown
(Can) Cervical
(Can) Middle
(Can) Apical
(M3) Crown
(M3) Cervical
(M3) Middle
(M3) Apical
Rib
E
Sample: EE3
F
Sample: NA59

Fig. 4 (a–f)

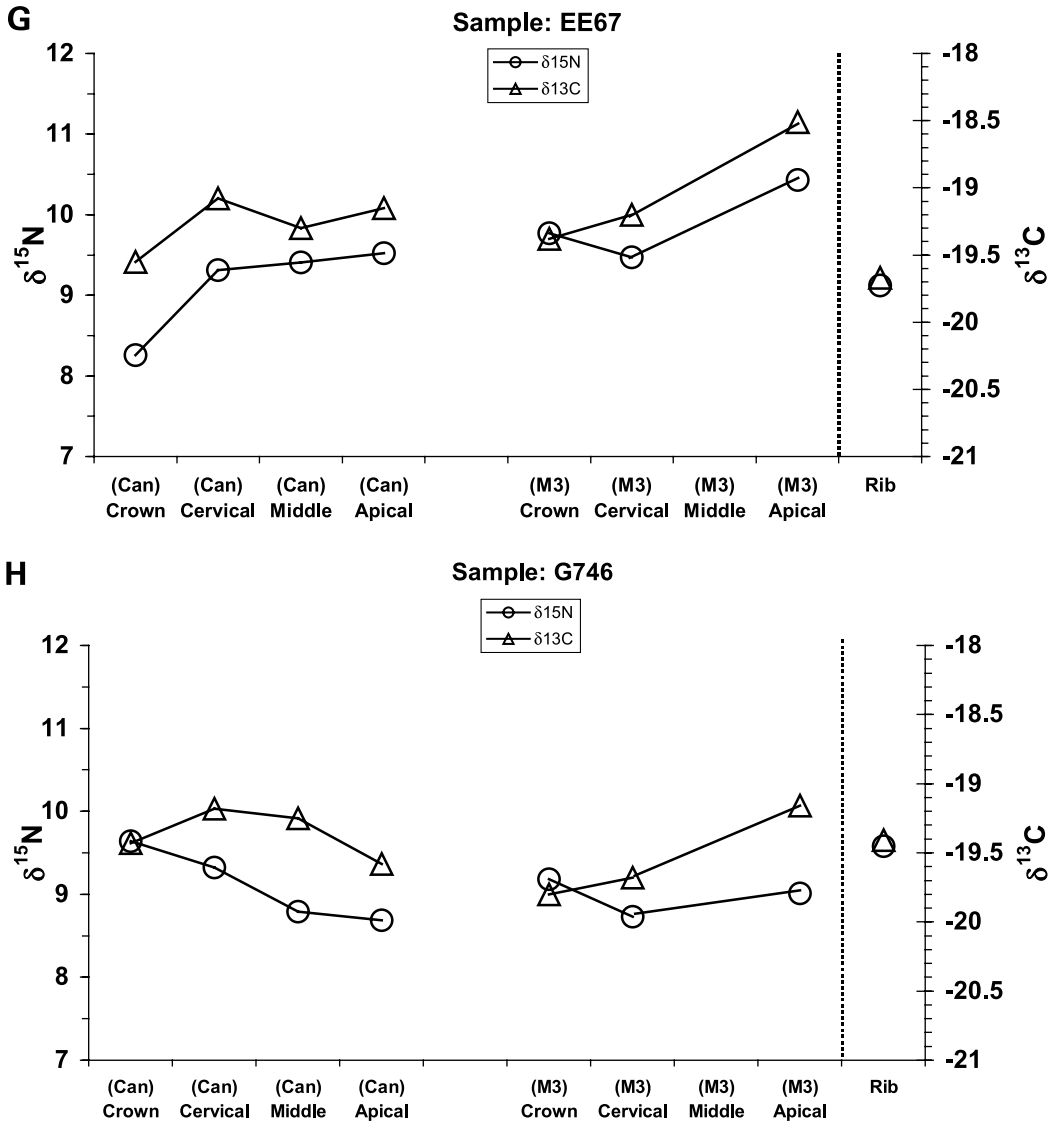


Fig. 4. (a–h). $\delta^{13}C$ and $\delta^{15}N$ values for dentine serial sections from permanent canines (Can), permanent third molars (M3) and ribs from the same individuals.

protein consumption versus plant protein consumption but has also been attributed to physiological and environmental factors [1,20,36,37]. Six of the canine crowns (EE36, G597, CN2, EE3, NA59, G746) are enriched in ^{15}N over the cervical halves of the roots, which is likely the result of breastfeeding. This is as expected as canine dentine development starts at about 5 months of age (Table 1a). Five of the rib samples (EE36, CN28, CN2, EE3, G746) have $\delta^{15}N$ values nearly equal to or greater than apical portions of the third molar roots (Fig. 4a, b, d, e, h), illustrating for these individuals that the consumption of animal protein was constant or had increased during the later part of life as rib collagen reflects the diet from approximately the last 5 to 10 years of life [39]. In contrast, both of the males studied (NA59, EE67) show a decrease in $\delta^{15}N$ from the apical halves of the third molar roots to the ribs by 1.1‰ and 1.3‰, respectively (Fig. 4f, g). This depletion in ^{15}N can likely be attributed to decreased animal protein consumption in later life. With such a small study (8 individuals) over such a large period of time (approximately 600 years), the present results clearly may not be representative of the entire Wharram Percy population, yet they show that the dietary habits of these inhabitants were not static but changed with age. Analyses of dental material from further skeletons would be necessary to establish if these dietary trends were representative of this community as a whole.

5. Conclusions

We have conducted a study on the deciduous and permanent dentine serial sections of human teeth, using material from the medieval village site of Wharram

Percy. For deciduous second molars and ribs, the following isotopic enrichment pattern was observed for both ^{13}C and ^{15}N: crown>cervical part of root>apical part of root>ribs. This pattern is the result of decreased breast milk consumption and an increased intake of weaning foods. After the age of weaning (2 years old), the mean difference between the dentine crowns and the ribs was $1.2 \pm 0.4‰$ for $\delta^{13}C$ and $3.2 \pm 0.8‰$ for $\delta^{15}N$. While this ^{13}C enrichment has been seen elsewhere [13,22,23,41], these results are unique as they can be clearly attributed to a trophic level effect of breast-feeding infants, as there were no known C_4 plants at Wharram Percy.

The comparison of the deciduous second molar crowns from individuals who died before weaning with those who survived past the age of weaning (about 2 years old) failed to find a dietary difference between the two age groups. While the sample size was small, this lack of a different feeding regime between those who survived beyond infancy/early childhood and those who did not suggests that earlier inferences concerning infant feeding patterns [28,31] based on analyses of non-survivors are likely to be a reasonable indication of usual feeding practices in this population rather than defining some abnormal, unsuccessful strategy. The serial sectioning of the permanent dentition indicated that there was little change in the sources of dietary carbon during life, but two individuals seem to show evidence of minor inputs of marine protein in their diets. The $\delta^{15}N$ results were more variable with some individuals likely consuming equal or more animal protein in later life.

Finally, this research has demonstrated the potential for using isotopic results from human dentine serial sections to deduce individual dietary habits. Future work in this area should focus on the sectioning of teeth along the lines of growth for dentine. As the formation rates for teeth are well characterized [19], the meticulous sampling of dentine along the lines of growth has the ability to determine weaning patterns and dietary habits at the refined level of the individual. Ideally, these dentine $\delta^{13}C$ and $\delta^{15}N$ measurements should be combined with enamel carbonate $\delta^{13}C$ and $\delta^{18}O$ results so that fuller isotopic life histories of past populations can be developed and interpreted.

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